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Nigella sativa Oil Has Significant Repairing Ability of Damaged Pancreatic Tissue Occurs in Induced Type 1 Diabetes Mellitus

Afaf Jamal Ali Hmza, Effat Omar, Ariza Adnan and Muhamed T. Osman

Centre of Pathology, Diagnostic and Research Laboratories, Faculty of Medicine,
Universiti Teknologi MARA (UiTM), Sg. Buloh Campus, 47000 Sg Buloh, Selangor, Malaysia

Abstract: Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease that impairs production of insulin. The disruption of insulin synthesis is caused by an autoimmune destruction of pancreatic islet cells. *Nigella sativa* oil (NSO) was known as hypoglycemic agent in both types of diabetes but little known about its ability of repairing the pancreatic damage occurred in T1DM. By intraperitoneal injection of a single dose of streptozotocin (STZ) (65 mg/kg), T1DM was induced in overnight fasted 24 rats. They were equally divided into four groups as following; (1) control group; (2) diabetic non treated group, (3) and (4) groups were treated with different doses of NSO (0.2 and 0.4 ml/kg) respectively, for a period of 30 consecutive days. Blood glucose was tested every morning through the experimental period. After completion the experimental protocol, blood samples were collected and serum insulin was assayed using ELISA. The pancreatic tail was dissected and kept in 10% formalin. The samples were processed using a tissue processor for histological study after H and E staining. The control group showed normal cells in pancreatic islet of Langerhans. The diabetic group with no treatment showed shrunken islets of Langerhans displaying degenerative and necrotic changes. Meanwhile, the treatment with low dose NSO protected the majority of cells in the islet of Langerhans, however the high dose NSO treatment showed a similar morphology as in normal control group (GA), so that resulted in significant elevation of serum insulin level ($p<0.005$). The data suggests that NSO treatment has a therapeutic effect against STZ induced T1DM rats.

Key words: *Nigella sativa* oil · Type1 diabetes mellitus · Blood glucose · Serum insulin · Pancreas

INTRODUCTION

Type 1 Diabetes mellitus or insulin depending diabetes mellitus (T1DM) is the most severe form of diabetes mellitus. According to WHO it accounts approximately 5-10% of all diabetic cases around the world and usually appears during childhood [1]. T1DM is caused by an autoimmune destruction of pancreatic β-cells, resulting in absolute deficiency in insulin production [2]. There are increase evidences that diabetes is combined by increase production of free radicals and reduction of antioxidant defense leading to development of diabetes ant its complications [3]. Many mechanisms seem to be involved in the initiation of oxidative stress which has been reported in experimental diabetes animals as well as in T1DM [4]. Oxidative stress is involved in the origin of type 1 diabetes especially through the destruction of pancreatic β cells [5].

Streptozotocin (STZ) is the most commonly used diabetogenic agent in the experimental animals to produce type 1 diabetes [6]. A single dose of 65mg/kg of STZ are able to induced T1DM to experimental animals [6-7].

More than 1000 different plants have been described for traditional treatment of diabetes [8]. Among these; the *Nigella sativa* (NS) which is a spice plant also knows as (black seed) has been used as an herbal medicine for more than 2000 years by different cultures to treat and prevent several diseases and illness. [9]. *NS* seeds have been reported to possess many biological activities including anti-inflammatory [10], anti-tumor [11], antihypertensive [12] and hypoglycemic properties [13, 14]. Moreover, many researchers have reported results supporting its potential value [15, 16]. *NS* is of great therapeutic benefit in diabetic individuals and those with glucose intolerance, as it accentuates glucose-induced secretion of insulin, besides having a negative impact on glucose absorption

Corresponding Author: Muhamed T. Osman, Centre of Pathology, Diagnostic and Research Laboratories, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sg. Buloh Campus, 47000 Sg Buloh, Selangor, Malaysia.

from the intestinal mucosa [17]. *NS* was proved it attenuates the damage to β-cells of the pancreas following exposure to toxic elements such as cadmium [18]. Recently, it has been investigated that the effects of *NS* oil (NSO) on some physiological parameters in streptozotocin (STZ)-induced diabetic rats. STZ induced diabetic rats showed a highly significant increase in the levels of blood glucose compared to the controls. Administration of *NSO* to diabetic rats resulted in a significant decrease in blood glucose levels after three weeks compared to untreated diabetic rats, indicated that the oil of *NS* possess hypoglycaemic effect in STZ-induced diabetic rats [19].

Many studies have been carried out to evaluate the role of *NSO* for management of diabetes [20], but the exact antidiabetic mechanism is not yet established regarding type 1 diabetes mellitus, however, in this present study the effect of *NSO* on the reversal process of histological damage caused by the disease process of IDDM were evaluated.

MATERIALS AND METHODS

Experimental Animals: Twenty four Male Sprague-Dawley rats with an average weight of 150-250g and an average age of 12-16 weeks were used throughout the experiments. The animals were obtained from Nano Life Quest Company. Ethical clearance for performing the experiment on animals was approved by Animal Care and Use Committee (ACUC), Faculty of Medicine, Universiti Teknologi MARA UiTM (ACUC-2/11). The rats were acclimatized for a period of 21 days in the Laboratory Animals Care Unit (LACU), Faculty of Medicine, Sg Buloh Campus, Universiti Teknologi MARA (UiTM) Malaysia. A standard environmental condition such as temperature (20-22°C), relative humidity (45-55%) and 12 hrs. dark/light cycles was maintained. The animals were fed daily with rodent pellet diet and tap water ad-libitum under strict hygienic conditions.

Chemicals: The Streptozotocin (STZ) (2-deoxy-2-({[methyl (nitroso) amino] carbonyl} amino)-β-D-glucopyranose) used in the present study was purchased from Sigma, Germany. The *Nigella sativa* oil was produced by Kausar, Iran. It is a pure preparation, with no additive. The oil was administered once a day by intraperitoneal (i.p) injection at doses of either 0.2 ml/kg or 0.4 ml/kg for 30 days.

Induction of Type 1 Diabetes and Experimental Design: T1DM was induced to overnight fasted animals by intraperitoneal injection of a single dose of STZ (65 mg/kg body weight) to all animal groups except the normal control group (25). STZ was dissolved in sodium citrate buffer solution (pH 4.5) immediately before use. The rats with blood glucose above 13.9 mmol/L (250 mg/dL), which lasted for at least three days, were considered as type 1 diabetic rat [21-22, 25].

The experimental animal groups were divided into 4 groups (6 rats each). The groups are: Group A (Normal control group received 65mg/bw sodium citrate buffer), Group B (Diabetic control group treated with STZ only 65 mg/kg), Group C (Diabetic rats received i.p. *NSO* low dose 0.2 ml/kg) and Group D (Diabetic rats received i.p. *NSO* high dose 0.4 ml/kg). Blood glucose was tested every morning (at 8 am) through the experimental period (30 days). The blood glucose level was tested by using glucometer purchased from (Roche, USA).

Laboratory Tests: Blood samples were collected from overnight fasted rats, after completion of 30 days of experimental protocols. The animals were anesthetized in a chamber containing diethyl ether. Blood was collected from the heart by the cardiac puncture. Immediately after collection, the blood was transferred into fresh tube and centrifuged at 3000 rpm for 10 minutes. The sera were stored at –80 °C until analysis. Serum was assayed for insulin level by using enzyme-linked immunosorbent assay (ELISA) (USCNK, CHINA).

The animals were dissected after scarification and the pancreas was taken for histology. This organ was placed in 10% formalin for fixation. Paraffin was used to embedding the pancreas and then stained with hematoxylin and eosin (H and E). The preparations were evaluated by Olympus multiheaded microscope and photographed by camera (Optiphot 2; Nikon, Tokyo, Japan).

Statistical Analysis: Data were analyzed by comparing values for different treatment groups with the values for the positive and negative control groups. Results are expressed as mean ± SD. The significant differences among values were analyzed using by one way analysis of variance (ANOVA) carried out by SPSS 16 software followed coupled with post-hoc least a p-value of < 0.05 was considered as statistically significance.

RESULTS AND DISCUSSION

Biochemical Results: In this study we had evaluated the effect of *NSO* on daily blood glucose and serum levels by using two different doses during 4 weeks of experimental.

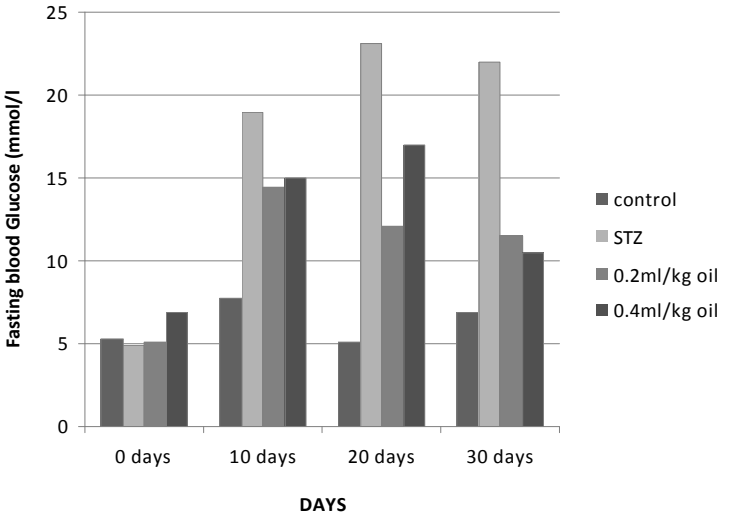

Fig. 1: Effects of *NSO* administration on Blood Glucose level. GA; control group, GB; untreated diabetic rats; GC, diabetic rats treated with (0.2 ml/kg) *NSO*. GD, diabetic rats treated with (0.4 ml/kg) *NSO*. Data are expressed as mean ±SD.

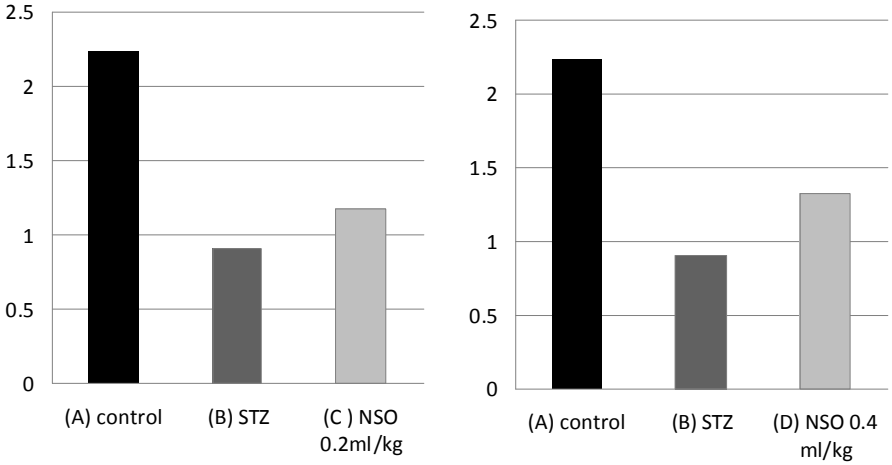

Fig. 2: Effects of *NSO* administration on level of Insulin production. GA; control group, GB; untreated diabetic rats; GC, diabetic rats treated with (0.2 ml/kg) *NSO*. GD, diabetic rats treated with (0.4 ml/kg) *NSO*. Data are expressed as mean ±SD.

After 10 days treatment with *NSO* 0.2 ml/kg, there was a slight, but non-significant decrease in blood glucose levels compared with those in the untreated diabetic group (Group B). Administration of *NSO* (0.2ml/kg) resulted in a significant lowering of elevated blood glucose levels after 30 days treatment compared with those in the untreated diabetic group ($p > 0.05$). Moreover, treatment of diabetic rats with *NSO* (0.4 ml/kg) resulted in a significant decrease in blood glucose levels compared with the untreated diabetic group after 10 days treatment. After 30 days, blood glucose levels had decreased to levels that did not differ significantly from basal levels seen in the control group ($p > 0.05$) (Figure 1). The present study has clearly shown that *NSO* at two different doses (0.2 - 0.4 ml/kg) cause a significant increase in serum insulin level after 30 days of treatment ($p > 0.05$) (Figure 2).

Histological Results: In normal control rat group (GA) the histological sections showed normal pancreatic structure. The islet of Langerhans appeared regular in shape surrounded by thin capsule of connective tissue. The clusters of cells are embedded in the pancreatic exocrine tissue. The cells are polygonal cells on shape and have regular nuclei (Figure 3.a). However, in STZ diabetic rats group with no treatment (GB), the findings on histologic sections of pancreatic tissues were degenerative, necrotic changes and shrinkage in the islets of Langerhans.

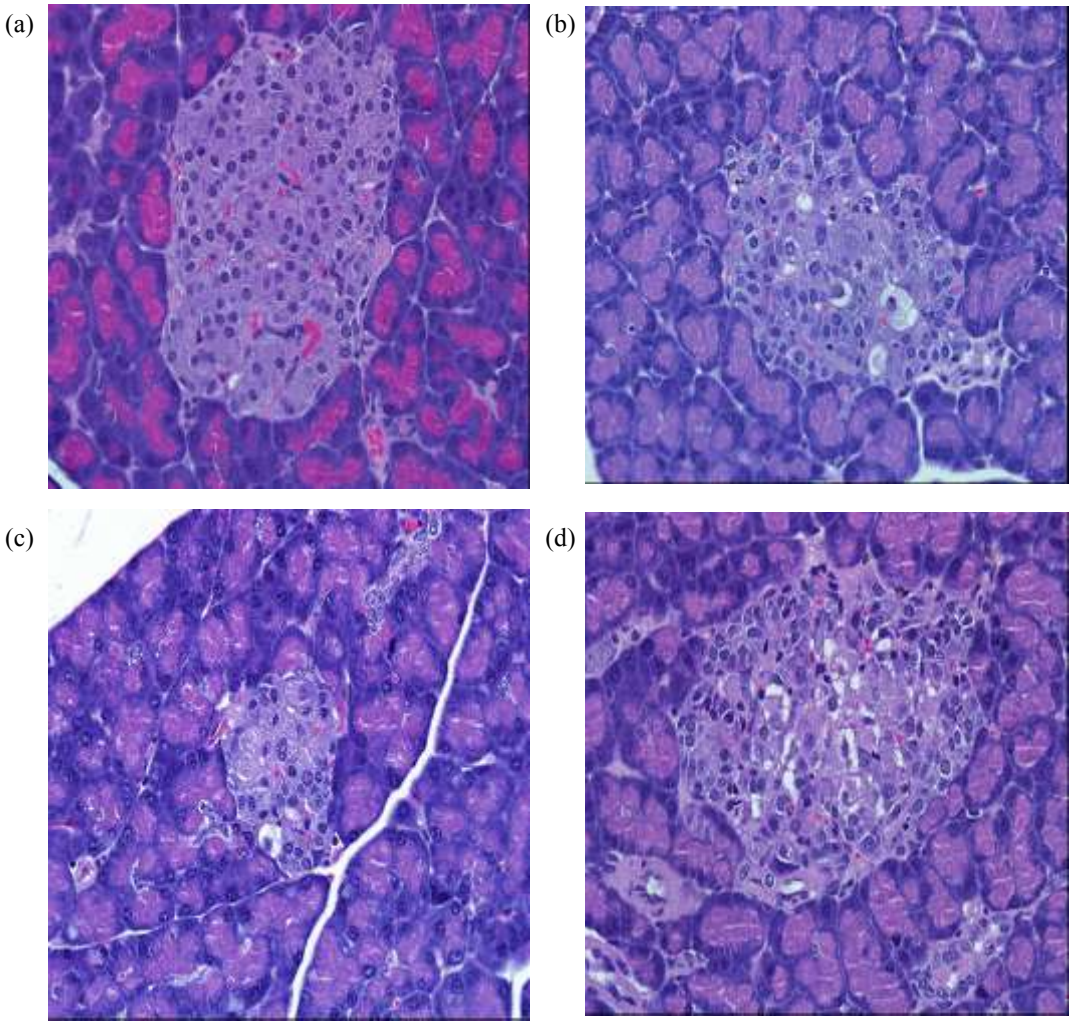

Fig. 3: a. Control group, b. STZ Diabetic group, c. 0.2 ml/kg (NSO) treated, d. 0.4 ml/kg (NSO) treated group.

The islets were relatively small, atrophied and showed a reduction in the number of polygonal islet cells. The nucleuses of necrotic cells are pynotic. The atrophied and degenerated cells show hydropic degeneration and loss of granules within the cytoplasm. The cytoplasm is dark and esonophilic (Figure 3.b).

In (GC) diabetic animals treatment with 0.2 ml/kg *NSO*, the histological sections showed that the islets were relatively small in size and irregular in shape compared with normal control (Figure 3.c). There were less hydropic degeneration, degranulation and necrosis in the islet cells. More viable polygonal islet cells were observed compared with in GB. Meanwhile, islets of Langerhans of (GD) rats treated with a high dose of *NSO* (0.4 ml/kg), showed similar appearance as in GA, there were more viable cells present and less hydropic degeneration, degranulated and necrosis compared with GB. The polygonal islet cells appeared with nuclei of various sizes (Figure 3.d).

DISCUSSION

The present study have shown that *NSO* produced a significant, consistent and time-dependent decrease in blood glucose levels and elevate insulin level production in STZ-T1DM induced diabetic rats compared with the untreated diabetic animals. The hypoglycemic effect observed in the present study became more significant after daily administration of the oil for one month to the diabetic animals. These results were consistent with other studies like Houcher *et al.,* 2007, who showed that the use of the commercial oil at a dose of 2.5 mL/ kg per day for 25 days significantly reduced blood glucose [5]. Data from other experimental study reported that blood glucose lowering effect of black seed oil was due to improved insulin insensitivity in diabetic rats [23]. A study done by other investigators reported that treatment with *NSO* commenced 6 weeks after induction of diabetes at a dose

of 400 mg/kg body weight by gastric lavage reduced blood glucose after the first, second, third and fourth weeks of treatment. They indicated that the hypoglycaemic effect of *NSO* is due to, at least in part, a decrease in hepatic gluconeogenesis [24].

In the present study we proved that NSO has significant increase on serum insulin levels, this result may be due to the effect of thymoqionone, a major component of *NSO*, These results are consistent with pervious study that have shown that daily gastric administration of 80 mg/ kg thymoquinone, for 45 days produces significant increase in insulin levels in STZ-diabetic rats [25]. From *in vitro* study, scientific investigations to the isolated pancreatic Islets of Langerhans have proven that the *N. sativa* extract causes direct stimulation of insulin release [26].

Many researchers have been focusing their studies to investigate various plants for their antidiabetic effect and have shown ability to prevent degenerative and metabolic effects in STZ induced diabetic animal models [27, 28]. Our study clearly indicated that, in IDDM diabetic rats, many histological changes were observed in the pancreatic islet as compared with the control. The islets were small and atrophied as well as the degenerative, necrotic changes and shrinking of the islets of Langerhans vaculation of cytoplasm and degranulations were observed. Our findings consistent with the results of several studies which detected same histological changes [29, 30].

In the present study, *NSO* treated group with 0.2 ml/kg did not ameliorate the destructive effect of STZ on islet cells. The histological sections showed that the islets were relatively small size and irregular in shape compared with control and there were light hydropic degeneration, degranulation and necrosis in the remaining cells. However, in treated rats with *NSO* at high dose of 0.4 ml/kg reduced the severity of degenerative and necrotic changes in islets of Langerhans. These protective effects may be attributed to the antioxidant properties of the *N. sativa.*

Results of current study were consistent with pervious study to Kanter *et al.,* (2004) who showed that the use of *NSO* reduced the severity of degenerative and necrotic changes in islets of STZ-diabetic rats [31]. Sections of pancreatic tissues of treated rats with NSO stained with haematoxylin and eosin showed that the majority of cells had light degeneration compared to the diabetic group with no treatment. Findings from the other study, suggested that the active ingredient thymoquinone is the most effective against STZ diabetes as its administration ameliorated most of the pathological changes [32].

In conclusion, the intraepithelial treatment with low dose *NSO* protected the majority of cells in the islet of Langerhans, however the high dose of *NSO* treatment showed a similar morphology as in normal control group, so that resulted in significant elevation of serum insulin level. The data suggested that *NSO* treatment has a therapeutic effect against induced T1DM rats.

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